L4 ANSWER 1 OF 3 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1993:389645 BIOSIS
DN PREV199396064945
TI **Aflagellated** mutants of **Helicobacter pylori** generated by genetic transformation of naturally competent strains using transposon shuttle mutagenesis.
AU Haas, Rainer (1); Meyer, Thomas F.; Van Putten, Jos P. M.
CS (1) Max-Planck-Inst. Biologie, Abteilung Infektionsbiologie, Spemannstrasse 34, D-7400 Tuebingen Germany
SO Molecular Microbiology, (1993) Vol. 8, No. 4, pp. 753-760. ISSN: 0950-382X.
DT Article
LA English
AB Three out of 10 **Helicobacter pylori** clinical isolates were found to be naturally competent for genetic transformation to streptomycin resistance by chromosomal DNA extracted from a spontaneous streptomycin-resistant **H. pylori** mutant. The frequency of transformation varied between 5 times 10-4 and 4 times 10-6, depending on the **H. pylori** isolate used. Transposon shuttle mutagenesis based on this natural competence was established using the flagellin gene flaA as the target. The cloned flaA gene was interrupted by insertion of TnMax1, a mini-Tn1721 transposon carrying a modified chloramphenicol-acetyltransferase gene, the cat-GC cassette. Natural transformation of competent **H. pylori** strains with plasmid constructs harbouring a cat-GC-inactivated flaA gene resulted in chloramphenicol-resistant transformants at an average frequency of 4 times 10-5. Southern hybridization experiments confirmed the replacement of the chromosomal **H. pylori** flaA gene by the cat-inactivated cloned gene copy via homologous recombination resulting in allelic exchange. Phenotypic characterization of the mutants demonstrated the absence of flagella under the electron microscope and the loss of bacterial motility. Immunoblots of cell lysates of the **H. pylori** mutants with an antiserum raised against the C-terminal portion of recombinant **H. pylori** major flagellin (FlaA) confirmed the absence of the 54 kDa FlaA protein. This efficient transposon shuttle mutagenesis procedure for **H. pylori** based on natural competence opens up new possibilities for the genetic assessment of putative **H. pylori** virulence determinants.

L7 ANSWER 1 OF 22 USPATFULL
AN 1999:50284 USPATFULL
TI Vaccines comprising enhanced antigenic helicobacter spp.
IN Pace, John Lee, Germantown, MD, United States
Walker, Richard Ives, Gaithersburg, MD, United States
Frey, Steven Michael, Germantown, MD, United States
PA Antex Biologics, Inc., Gaithersburg, MD, United States (U.S. corporation)
PI US 5897475 19990427
AI US 1995-538544 19951003 (8)
RLI Continuation-in-part of Ser. No. US 1994-318409, filed on 5 Oct 1994, now abandoned
DT Utility
LN.CNT 2096
INCL INCLM: 435/252.100
INCLS: 424/093.400; 424/184.100; 424/282.100
NCL NCLM: 435/252.100
NCLS: 424/093.400; 424/184.100; 424/282.100
IC [6]
ICM: A01N063-00
ICS: A61K039-38; A61K045-00; C12N001-20
EXF 435/252.2; 435/822; 435/252.1; 424/184.1; 424/93.4; 424/282.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 11 OF 22 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1995:290361 BIOSIS
DN PREV199598304661
TI Cloning, Expression, and Mutagenesis of the **H. pylori flbA** Gene - a Homolog of the lcrD/**flbF** Family of Genes Associated with Motility and Virulence.
AU Suerbaum, S. (1); Schmitz, A. (1); Josenhans, C. (1); Labigne, A.
CS (1) Med. Microbiol. Immunol., Ruhr Univ., Bochum Germany
SO Abstracts of the General Meeting of the American Society for Microbiology, (1995) Vol. 95, No. 0, pp. 181.
Meeting Info.: 95th General Meeting of the American Society for Microbiology Washington, D.C., USA May 21-25, 1995
ISSN: 1060-2011.
DT Conference
LA English

Cpst April 7 '95

July 4, 95

Suerbaum

others

L7 ANSWER 9 OF 22 USPATFULL
AN 97:96762 USPATFULL
TI Methods for producing enhanced antigenic campylobacter bacteria and vaccines
IN Pace, John Lee, Germantown, MD, United States
Walker, Richard Ives, Gaithersburg, MD, United States
Frey, Steven Michael, Germantown, MD, United States
PA Antex Biologics, Inc., Gaithersburg, MD, United States (U.S. corporation)
PI US 5679564 19971021
AI US 1995-538545 19951003 (8)
RLI Continuation-in-part of Ser. No. US 1994-318409, filed on 5 Oct 1994, now abandoned
DT Utility
LN.CNT 2162
INCL INCLM: 435/252.100
INCLS: 424/093.400; 424/184.100; 424/282.100
NCL NCLM: 435/252.100
NCLS: 424/093.400; 424/184.100; 424/282.100
IC [6]
ICM: A01N063-00
ICS: A61K039-38; A61K045-00; C12N001-20
EXF 435/252.1; 435/822; 424/93.4; 424/184.1; 424/282.1
C

L7 ANSWER 8 OF 22 USPATFULL
AN 97:99189 USPATFULL
TI Methods for producing enhanced antigenic shigella bacteria and vaccines comprising same
IN Pace, John Lee, Germantown, MD, United States
Walker, Richard Ives, Gaithersburg, MD, United States
Frey, Steven Michael, Germantown, MD, United States
PA Antex Biologics, Inc., Gaithersburg, MD, United States (U.S. corporation)
PI US 5681736 19971028
AI US 1995-538543 19951003 (8)
RLI Continuation-in-part of Ser. No. US 1994-318409, filed on 5 Oct 1994, now abandoned
DT Utility
LN.CNT 2158
INCL INCLM: 435/252.100
INCLS: 424/093.400; 424/184.100; 424/282.100
NCL NCLM: 435/252.100
NCLS: 424/093.400; 424/184.100; 424/282.100
IC [6]
ICM: A01N063-00
ICS: A61K039-00; A61K045-00; C12N001-20
EXF 435/252.1; 435/822; 424/93.4; 424/184.1; 424/282.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 12 OF 22 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1995:471417 BIOSIS
DN PREV199598485717
TI The **H. pylori** flagellar biosynthesis regulatory protein **FlbA** affects the expression of flagellar components of the transcriptional level and is probably a membrane protein.
AU Schmitz, Andre; Josenhans, Christine; Suerbaum, Sebastian
CS Med. Microbiol. Immunol., Ruhr-Univ., D-44780 Bochum Germany
SO Gut, (1995) Vol. 37, No. SUPPL. 1, pp. A62.
Meeting Info.: VIIIth International Workshop on Gastro-duodenal Pathology and Helicobacter pylori Edinburgh, Scotland, UK July 7-9, 1995
ISSN: 0017-5749.
DT Conference
L

L7 ANSWER 8 OF 12 MEDLINE
AN 96294750 MEDLINE
DN 96294750
TI Colonization of gnotobiotic piglets by **Helicobacter pylori** deficient in two flagellin genes.
AU Eaton K A; Suerbaum S; Josenhans C; Krakowka S
CS Ohio State University, Columbus 43210, USA.
NC R29 DK 45340 (NIDDK)
SO INFECTION AND IMMUNITY, (1996 Jul) 64 (7) 2445-8.
Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199611
AB Helicobacterpylori possesses two flagellin molecules, MA, the major species, and FlaB, which is expressed in minor amounts. This study sought to determine if one or both flagellin species are necessary for colonization or persistence by H. pylon. Thirty-six gnotobiotic piglets from six litters were given one of four isogenic strains of H. pylon orally. The bacterial strains used were strain N6, the wild type, which produced both FlaA and FlaB and was fully motile; N6flaB::km, which produced FlaA but not FlaB and was weakly motile; N6flaA::km, which expressed FlaB but not FlaA and was **nonmotile**; and N6flaA::cat/flaB::km, which produced neither flagellin and was **nonmotile**. Strain N6 colonized all piglets and persisted for 2, 4, and 10 days after inoculation. Both N6flaA::km and N6flaB::km colonized for 2 and 4 but not 10 days, and colonization was weak. N6flaA::cat/flaB:: km colonized for 2 days but did not persist for 4 or 10 days after inoculation. These findings demonstrate that both flagellin species are necessary for full colonization by H. pylon. Colonization for up to 4 days is possible in the absence of either flagellin species but not both.

L4 ANSWER 2 OF 15 MEDLINE
AN 95048133 MEDLINE
DN 95048133
TI Studies on gastric mucosal cell injury induced by Helicobacter pylori.
AU Mitani-Ehara S
CS Third Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo, Japan..
SO HOKKAIDO IGAKU ZASSHI. HOKKAIDO JOURNAL OF MEDICAL SCIENCE, (1994 Jul) 69 (4) 836-46.
Journal code: GA9. ISSN: 0367-6102.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
LA Japanese
FS Priority Journals
EM 199502
AB The cause of gastric mucosal cell injury induced by Helicobacter pylori (H. pylori) was investigated in vitro using gastric mucosal cells derived from the stomach of male Japanese white rabbits. In order to evaluate the contribution of potent urease activity of H. pylori to gastric mucosal cell injury, supernatant of H. pylori bacterial pellet solubilized in a 1.0% solution of **n-octyl-glucoside**, the H. pylori **extracts**, was added to the rabbit gastric mucosal cell suspension. Cell injury was expressed by LDH release into the extracellular fluid of gastric mucosal cell suspension after 30 minutes incubation at 37 degrees C. Treatment of cells by H. pylori extracts (final concentration of 0.54 mg/ml) together with urea (final concentration at 50 mM) showed a high LDH release into the extracellular fluid suggesting definite gastric mucosal cell injury. Elevation of ammonia concentration and that of extracellular fluid pH were also observed by the treatment, whereas H. pylori extracts alone and urea solution alone did not. The ammonia concentration of extracellular fluid and LDH release were distinctly elevated in accord with increasing amount of H. pylori extracts under the existence of 50 mM urea. The degree of LDH release from gastric mucosal cell by H. pylori extracts under the existence of urea was similar to that induced by the administration of the same amount of exogenous ammonia. The addition of acetohydroxamic acid (AHA), a potent specific urease inhibitor, remarkably inhibited dose dependently the ammonia production, the elevation of pH of extracellular fluid and LDH release induced by H. pylori extracts under the presence of urea. These results suggest that the ammonia produced by potent urease activity of H. pylori under the presence of urea played an important role in the pathogenesis of gastric mucosal cell injury.

L3 ANSWER 1 OF 2 MEDLINE
AN 1999242780 MEDLINE
DN 99242780
TI Molecular characterization of a flagellar export locus of Helicobacter pylori.
AU Porwollik S; Noonan B; O'Toole P W
CS Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand.
SO INFECTION AND IMMUNITY, (1999 May) 67 (5) 2060-70.
Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-U75584
EM 199907
EW 19990704
AB Motility of Helicobacter species has been shown to be essential for successful colonization of the host. We have investigated the organization of a flagellar export locus in Helicobacter pylori. A 7-kb fragment of the H. pylori CCUG 17874 genome was cloned and sequenced, revealing an operon comprising an open reading frame of unknown function (ORF03), essential housekeeping genes (ileS and murB), flagellar export genes (fliI and fliQ), and a homolog to a gene implicated in virulence factor transport in other pathogens (virB11). A promoter for this operon, showing similarity to the Escherichia coli sigma70 consensus, was identified by primer extension. Cotranscription of the genes in the operon was demonstrated by reverse transcription-PCR, and transcription of virB11, fliI, fliQ, and murB was detected in human or mouse biopsies obtained from infected hosts.
The genetic organization of this locus was conserved in a panel of **H. pylori** clinical isolates. Engineered fliI and fliQ mutant strains were completely **aflagellate** and nonmotile, whereas a virB11 mutant still produced flagella. The fliI and fliQ mutant strains produced reduced levels of flagellin and the hook protein FlgE. Production of OMP4, a member of the outer membrane protein family identified in H. pylori 26695, was reduced in both the virB11 mutant and the fliI mutant, suggesting related functions of the virulence factor export protein (VirB11) and the flagellar export component (FliI).

L3 ANSWER 2 OF 2 MEDLINE
AN 97375061 MEDLINE
DN 97375061
TI A flagellar-specific ATPase (FliI) is necessary for flagellar export in Helicobacter pylori.
AU Jenks P J; Foynes S; Ward S J; Constantinidou C; Penn C W; Wren B W
CS Department of Medical Microbiology, St. Bartholomew's Hospital, West Smithfield, London, UK.
SO FEMS MICROBIOLOGY LETTERS, (1997 Jul 15) 152 (2) 205-11.
Journal code: FML. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-Y08620
EM 199710

AB Although flagellar motility is essential for the colonisation of the stomach by Helicobacter pylori, little is known about the regulation of flagellar biosynthesis in this organism. We have identified a gene in H. pylori, designated fliI, whose deduced amino acid sequence revealed extensive homology with the FliI/LcrB/InvC family of proteins which energise the export of flagellar and other virulence factors in several bacterial species. An isogenic mutant of fliI was non-motile and synthesised reduced amounts of flagellin and hook protein subunits. The majority (> 99%) of mutant cells were completely **aflagellate**. These results suggest that FliI is a novel ATPase involved in flagellar export in **H. pylori**.

L5 ANSWER 1 OF 1 MEDLINE
AN 2000026809 MEDLINE
DN 20026809
TI Molecular cloning and characterization of the Helicobacter pylori fliD gene, an essential factor in flagellar structure and motility.
AU Kim J S; Chang J H; Chung S I; Yum J S
CS Mogam Biotechnology Research Institute, Koosung-myon, Yongin-city, Kyonggi-do 449-910, Korea.. jsyum@kgcc.co.kr
SO JOURNAL OF BACTERIOLOGY, (1999 Nov) 181 (22) 6969-76.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-U82981
EM 200002
EW 20000204
AB Helicobacter pylori colonizes the human stomach and can cause gastroduodenal disease. Flagellar motility is regarded as a major factor in the colonizing ability of H. pylori. The functional roles of flagellar structural proteins other than FlaA, FlaB, and FlgE are not well understood. The fliD operon of H. pylori consists of flaG, fliD, and fliS genes, in the order stated, under the control of a sigma(28)-dependent promoter. In an effort to elucidate the function of the FliD protein, a hook-associated protein 2 homologue, in flagellar morphogenesis and motility, the fliD gene (2,058 bp) was cloned and isogenic mutants were constructed by disruption of the fliD gene with a kanamycin resistance cassette and electroporation-mediated allelic-exchange mutagenesis. In the fliD mutant, morphologically abnormal flagellar appendages in which very little filament elongation was apparent were observed. The fliD mutant strain was completely **nonmotile**, indicating that these abnormal flagella were functionally defective. Furthermore, the isogenic fliD mutant of **H. pylori** SS1, a mouse-adapted strain, was not able to colonize the gastric mucosae of host mice. These results suggest that H. pylori FliD is an essential element in the assembly of the functional flagella that are required for colonization of the gastric mucosa.

L7 ANSWER 1 OF 12 MEDLINE
AN 2000458522 MEDLINE
DN 20416244
TI Mutational analysis of genes encoding the early flagellar components of **Helicobacter pylori**: evidence for transcriptional regulation of flagellin A biosynthesis.
AU Allan E; Dorrell N; Foynes S; Anyim M; Wren B W
CS Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, United Kingdom.
SO JOURNAL OF BACTERIOLOGY, (2000 Sep) 182 (18) 5274-7. Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200012
EW 20001201
AB We investigated the roles of fliF, fliS, flhB, fliQ, fliG, and fliI of **Helicobacter pylori**, predicted by homology to encode structural components of the flagellar basal body and export apparatus. Mutation of these genes resulted in **nonmotile**, nonflagellate strains. Western blot analysis showed that all the mutants had considerably reduced levels of both flagellin subunits and of FlgE, the flagellar hook protein. RNA slot blot hybridization showed reduced levels of flaA mRNA, indicating that transcription of the major flagellin gene is inhibited in the absence of the early components of the flagellar-assembly pathway. This is the first demonstration of a checkpoint in **H. pylori** flagellar assembly.

L7 ANSWER 2 OF 12 MEDLINE
AN 2000404329 MEDLINE
DN 20359354
TI Switching of flagellar motility in **Helicobacter pylori** by reversible length variation of a short homopolymeric sequence repeat in fliP, a gene encoding a basal body protein.
AU Josenhans C; Eaton K A; Thevenot T; Suerbaum S
CS Institute of Hygiene and Microbiology, University of Wurzburg, D-97080 Wurzburg, Germany.
NC RO1 AI43643 (NIAID)
R29 DK-45340 (NIDDK)
SO INFECTION AND IMMUNITY, (2000 Aug) 68 (8) 4598-603. Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-AJ404379; GENBANK-AJ404380; GENBANK-AJ404381; GENBANK-AJ404382; GENBANK-AJ404383; GENBANK-AJ404384; GENBANK-AJ404385; GENBANK-AJ404386; GENBANK-AJ404387; GENBANK-AJ404388; GENBANK-AJ404389; GENBANK-AJ404390; GENBANK-AJ404391; GENBANK-AJ404392; GENBANK-AJ404393; GENBANK-AJ404394; GENBANK-AJ404395; GENBANK-AJ404396; GENBANK-AJ404397; GENBANK-AJ404398; GENBANK-AJ404399; GENBANK-AJ404400
EM 200010
EW 20001004
AB The genome of **Helicobacter pylori** contains numerous

simple nucleotide repeats that have been proposed to have regulatory functions and to compensate for the conspicuous dearth of master regulatory pathways in this highly host-adapted bacterium. **H. pylori** strain 26695, whose genomic sequence was determined by The Institute for Genomic Research (TIGR), contains a repeat of nine cytidines in the fliP flagellar basal body gene that splits the open reading frame in two parts. In this work, we demonstrate that the 26695(C9) strain with a split fliP gene as sequenced by TIGR was nonflagellated and **nonmotile**. In contrast, earlier isolates of strain 26695 selected by positive motility testing as well as pig-passaged derivatives of 26695 were all flagellated and highly motile. All of these motile strains had a C(8) repeat and consequently a contiguous fliP reading frame. By screening approximately 50,000 colonies of 26695(C9) for motility in soft agar, a motile revertant with a C(8) repeat could be isolated, proving that the described switch is reversible. The fliP genes of 20 motile clinical **H. pylori** isolates from different geographic regions possessed intact fliP genes with repeats of eight cytidines or the sequence CCCCACCC in its place. Isogenic fliP mutants of a motile, C(8) repeat isolate of strain 26695 were constructed by allelic exchange mutagenesis and found to be defective in flagellum biogenesis. Mutants produced only small amounts of flagellins, while the transcription of flagellin genes appeared unchanged. These results strongly suggest a unique mechanism regulating motility in **H. pylori** which relies on slipped-strand mispairing-mediated mutagenesis of fliP.

L7 ANSWER 3 OF 12 MEDLINE
AN 2000083056 MEDLINE
DN 20083056
TI Virulence factors of **Helicobacter pylori** affecting its gastric colonization in Mongolian gerbils.
AU Iwao E; Hirayama F; Takagi S; Yokoyama Y; Ikeda Y
CS Research Laboratories, Yoshitomi Pharmaceutical Industries, Fukuoka, Japan.
SO JOURNAL OF GASTROENTEROLOGY, (1999) 34 Suppl 11 47-54.
Journal code: BWP. ISSN: 0944-1174.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200004
EW 20000401
AB **Helicobacter pylori** is recognized to possess a number of virulence factors. We investigated the role of motility, vacuolating cytotoxin, and urease in gastric colonization by **H. pylori**. Mongolian gerbils (SPF, 7 weeks old) were challenged orally with a single administration of a 24-h culture broth of **H. pylori** and then were killed 6 and 26 weeks after challenge. Gastric colonization, severe gastritis, ulceration, and high levels of serum anti-**H. pylori** immunoglobulin G were observed in the gerbils challenged with strains motile in the semisolid medium (ATCC43504, HPY-127, HPY-204), but not in gerbils challenged with strains **nonmotile** in the medium (ATCC49503, HPY-205, HPY-206). Only strains ATCC43504, ATCC49503, HPY-204, and HPY-206 had vacuolating cytotoxin activity against HeLa and Vero cells. Thus, motile strains were able to colonize regardless of their vacuolating cytotoxin activities, and vacuolating cytotoxin was not associated with epithelial damage in the gastric mucosa. Furthermore, the phenotypic variants of strains with the ability to colonize that lacked either motility or urease activity lost their ability to colonize. In conclusion, motility and urease activity, but not vacuolating cytotoxin activity, are essential for gastric colonization by **H. pylori** in Mongolian gerbils.

L7 ANSWER 4 OF 12 MEDLINE

AN 2000080623 MEDLINE
DN 20080623
TI Performance of native and recombinant antigens for diagnosis of **Helicobacter pylori** infection.
AU Widmer M; de Korwin J D; Aucher P; Thiberge J M; Suerbaum S; Labigne A; Fauch`ere J L
CS Sanofi Diagnostic Pasteur, Marne-La-Coquette, France.
SO EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES, (1999 Nov) 18 (11) 823-6.
Journal code: EM5. ISSN: 0934-9723.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200003
EW 20000303
AB The aim of this study was to evaluate the performance of three antigenic preparations for serological diagnosis of **Helicobacter pylori** infection: (i) native antigens from **Helicobacter pylori** strain N6 or its **aflagellated** isogenic mutant N6flbA-, or an acellular extract (antigen AgFA) from a pool of six clinical strains; (ii) recombinant antigens consisting of CagA fused to MS2 polymerase and HspA or recombinant UreA and UreB fused to the maltose-binding protein, and (iii) the preparations provided with two commercial kits, the Cobas Core (Roche Diagnostic Systems, France) and the Pylori Stat (BioWhittaker, Belgium). All preparations were used in an enzyme immunoassay to test 92 sera from dyspeptic patients for whom the status of Helicobacter infection was established. Sensitivities were higher (90 to 100%) for the native antigens and the commercial kits than for the recombinant antigens. Specificities were higher than 90%, except with UreA + UreB (42%). The most useful antigens were those extracted from strains N6 and N6flbA-.

L7 ANSWER 5 OF 12 MEDLINE
AN 1999242831 MEDLINE
DN 99242831
TI Identification of virulence genes of **Helicobacter pylori** by random insertion mutagenesis.
AU Bijlsma J J; Vandenbroucke-Grauls C M; Phadnis S H; Kusters J G
CS Department of Medical Microbiology, Faculty of Medicine, Vrije Universiteit Amsterdam, The Netherlands.
NC CA67527 (NCI)
DK39045 (NIDDK)
SO INFECTION AND IMMUNITY, (1999 May) 67 (5) 2433-40.
Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199907
EW 19990704
AB The complete genome of the gram-negative bacterial pathogen **Helicobacter pylori**, an important etiological agent of gastroduodenal disease in humans, has recently been published. This sequence revealed that the putative products of roughly one-third of the open reading frames (ORFs) have no significant homology to any known proteins. To be able to analyze the functions of all ORFs, we constructed an integration plasmid for **H. pylori** and used it to generate a random mutant library in this organism. This integration plasmid, designated pBCalpha3, integrated randomly into the chromosome of **H. pylori**. To test the capacity of this library to identify virulence genes, subsets of this library were screened for urease-negative mutants and for **nonmotile** mutants. Three urease-negative mutants in a subset of 1,251 mutants (0.25%) and 5

nonmotile mutants in a subset of 180 mutants (2.7%) were identified. Analysis of the disrupted ORFs in the [redacted]ease-negative mutants revealed that two had disruptions of genes of the [redacted]ease locus, ureB and ureI, and the third had a disruption of a unrelated gene; a homologue of deaD, which encodes an RNA helicase. Analysis of the disrupted ORFs in the **nonmotile** mutants revealed one ORF encoding a homologue of the paralyzed flagellar protein, previously shown to be involved in motility in Campylobacter jejuni. The other four ORFs have not been implicated in motility before. Based on these data, we concluded that we have generated a random insertion library in **H. pylori** that allows for the functional identification of genes in **H. pylori**.

L7 ANSWER 6 OF 12 MEDLINE
AN 97386399 MEDLINE
DN 97386399
TI The flgE gene of Campylobacter coli is under the control of the alternative sigma factor sigma54.
AU Kinsella N; Guerry P; Cooney J; Trust T J
CS Department of Biochemistry and Microbiology, University of Victoria, British Columbia, Canada.
SO JOURNAL OF BACTERIOLOGY, (1997 Aug) 179 (15) 4647-53.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF004221
EM 199712
AB The flgE gene encoding the flagellar hook protein of Campylobacter coli VC167-T1 was cloned by immunoscreening of a genomic library constructed in lambdaZAP Express. The flgE DNA sequence was 2,553 bp in length and encoded a protein with a deduced molecular mass of 90,639 Da. The sequence had significant homology to the 5' and 3' sequences of the flgE genes of **Helicobacter pylori**, Treponema phagedenis, and Salmonella typhimurium. Primer extension analysis indicated that the VC167 flgE gene is controlled by a sigma54 promoter. PCR analysis showed that the flgE gene size and the 5' and 3' DNA sequences were conserved among C. coli and C. jejuni strains. Southern hybridization analyses confirmed that there is considerable sequence identity among the hook genes of C. coli and C. jejuni but that there are also regions within the genes which differ. Mutants of C. coli defective in hook production were generated by allele replacement. These mutants were **nonmotile** and lacked flagellar filaments. Analyses of flgE mutants indicated that the carboxy terminus of FlgE is necessary for assembly of the hook structure but not for secretion of FlgE and that, unlike salmonellae, the lack of flgE expression does not result in repression of flagellin expression.

L7 ANSWER 7 OF 12 MEDLINE
AN 97175520 MEDLINE
DN 97175520
TI Cloning and characterization of the **Helicobacter pylori** flbA gene, which codes for a membrane protein involved in coordinated expression of flagellar genes.
AU Schmitz A; Josenhans C; Suerbaum S
CS Ruhr-Universitat Bochum, Medizinische Mikrobiologie und Immunologie, Germany.
SO JOURNAL OF BACTERIOLOGY, (1997 Feb) 179 (4) 987-97.
Journal code: HH3. ISSN: 0021-9193.
CY United States

Petal 6/9/13

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-Y13395
EM 199705
AB Flagellar motility has been shown to be an essential requirement for the ability of **Helicobacter pylori** to colonize the gastric mucosa. While some flagellar structural components have been studied in molecular detail, nothing was known about factors that play a role in the regulation of flagellar biogenesis. We have cloned and characterized an **H. pylori** homolog (named flbA) of the lcrD/flbF family of genes. Many proteins encoded by these genes are known to be involved in flagellar biogenesis or secretion of virulence-associated proteins via type III secretion systems. The **H. pylori** flbA gene (2,196 bp) is capable of coding for a predicted 732-amino-acid, 80.9-kDa protein that has marked sequence similarity with other known members of the LcrD/FlbF protein family. An isogenic strain with a mutation in the flbA gene was constructed by disruption of the gene with a kanamycin resistance cassette and electroporation-mediated allelic exchange mutagenesis. The mutant strain expressed neither the FlaA nor the FlaB flagellin protein. The expression of the FlgE hook protein was reduced in comparison with the wild-type strain, and the extent of this reduction was growth phase dependent. The flbA gene disruption was shown to downregulate the expression of these flagellar genes on the transcriptional level. The flbA mutants were **aflagellate** and completely **nonmotile**. Occasionally, assembled hook structures could be observed, indicating that export of axial flagellar filament components was still possible in the absence of the flbA gene product. The hydrophilic part of the FlbA protein was expressed in Escherichia coli, purified, and used to raise a polyclonal rabbit antiserum against the FlbA protein. Western blot experiments with this antiserum indicated that the FlbA protein is predominantly associated with the cytoplasmic membrane in **H. pylori**. The antiserum cross-reacted with two other proteins (97 and 43 kDa) whose expression was not affected by the flbA gene disruption and which might represent further **H. pylori** homologs of the LcrD/FlbF protein family.

L7 ANSWER 8 OF 12 MEDLINE
AN 96294750 MEDLINE
DN 96294750
TI Colonization of gnotobiotic piglets by **Helicobacter pylori** deficient in two flagellin genes.
AU Eaton K A; Suerbaum S; Josenhans C; Krakowka S
CS Ohio State University, Columbus 43210, USA.
NC R29 DK 45340 (NIDDK)
SO INFECTION AND IMMUNITY, (1996 Jul) 64 (7) 2445-8.
Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199611
AB Helicobacterpylori possesses two flagellin molecules, MA, the major species, and FlaB, which is expressed in minor amounts. This study sought to determine if one or both flagellin species are necessary for colonization or persistence by H. pylon. Thirty-six gnotobiotic piglets from six litters were given one of four isogenic strains of H. pylon orally. The bacterial strains used were strain N6, the wild type, which produced both FlaA and FlaB and was fully motile; N6flaB::km, which produced FlaA but not FlaB and was weakly motile; N6flaA::km, which expressed FlaB but not FlaA and was **nonmotile**; and N6flaA::cat/flaB::km, which produced neither flagellin and was **nonmotile**. Strain N6 colonized all piglets and persisted for 2, 4, and 10 days after inoculation. Both N6flaA::km and N6flaB::km colonized for 2 and 4 but not 10 days, and colonization was weak. N6flaA::cat/flaB::km colonized for 2 days but did not persist for 4 or 10 days after inoculation. These findings demonstrate that both flagellin species are necessary for full colonization by H. pylon. Colonization for up to 4 days is possible in the absence of either flagellin species but not both.

July 96. post based on freezer prints

L7 ANSWER 9 OF 12 MEDLINE
AN 95286478 MEDLINE
DN 95286478
TI Comparative ultrastructural and functional studies of **Helicobacter pylori** and Helicobacter mustelae flagellin mutants: both flagellin subunits, FlaA and FlaB, are necessary for full motility in Helicobacter species.
AU Josenhans C; Labigne A; Suerbaum S
CS Medizinische Mikrobiologie und Immunologie, Ruhr-Universitat Bochum, Germany..
SO JOURNAL OF BACTERIOLOGY, (1995 Jun) 177 (11) 3010-20.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-L38478
EM 199509
AB Helicobacter mustelae causes chronic gastritis and ulcer disease in ferrets. It is therefore considered an important animal model of human **Helicobacter pylori** infection. High motility even in a viscous environment is one of the common virulence determinants of Helicobacter species. Their sheathed flagella contain a complex filament that is composed of two distinctly different flagellin subunits, FlaA and FlaB, that are coexpressed in different amounts. Here, we report the cloning and sequence determination of the flaA gene of H. mustelae NCTC12032 from a PCR amplification product. The FlaA protein has a calculated molecular mass of 53 kDa and is 73% homologous to the **H. pylori** FlaA subunit. Isogenic flaA and flaB mutants of H. mustelae F1 were constructed by means of reverse genetics. A method was established to generate double mutants (flaA flaB) of H. mustelae F1 as well as **H. pylori** N6. Genotypes, motility properties, and morphologies of the H. mustelae flagellin mutants were determined and compared with those of the **H. pylori** flaA and flaB mutants described previously. The flagellar organizations of the two Helicobacter species proved to be highly similar. When the flaB genes were disrupted, motility decreased by 30 to 40%. flaA mutants retained weak motility by comparison with strains that were devoid of both flagellin subunits. Weakly positive motility tests of the flaA mutants correlated with the existence of short truncated flagella. In H. mustelae, lateral as well as polar flagella were present in the truncated form. flaA flaB double mutants were completely **nonmotile** and lacked any form of flagella. These results show that the presence of both flagellin subunits is necessary for complete motility of Helicobacter species. The importance of this flagellar organization for the ability of the bacteria to colonize the gastric mucosa and to persist in the gastric mucus remains to be proven

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L7 ANSWER 10 OF 12 MEDLINE
AN 93323753 MEDLINE
DN 93323753
TI **Aflagellated** mutants of **Helicobacter pylori** generated by genetic transformation of naturally competent strains using transposon shuttle mutagenesis.
AU Haas R; Meyer T F; van Putten J P
CS Max-Planck-Institut fur Biologie, Abteilung Infektionsbiologie, Tubingen, Germany..
SO MOLECULAR MICROBIOLOGY, (1993 May) 8 (4) 753-60.
Journal code: MOM. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199310
AB Three out of 10 **Helicobacter pylori** clinical isolates were found to be naturally competent for genetic transformation to streptomycin resistance by chromosomal DNA extracted from a spontaneous streptomycin-resistant **H. pylori** mutant. The frequency of transformation varied between 5 x 10(-4) and 4 x 10(-6), depending on the **H. pylori** isolate used. Transposon shuttle mutagenesis based on this natural competence was established using the flagellin gene flaA as the target. The cloned flaA gene was interrupted by insertion of TnMax1, a mini-Tn1721 transposon carrying a modified chloramphenicol-acetyltransferase gene, the catGC cassette. Natural transformation of competent **H. pylori** strains with plasmid constructs harbouring a catGC-inactivated flaA gene resulted in chloramphenicol-resistant transformants at an average frequency of 4 x 10(-5). Southern hybridization experiments confirmed the replacement of the chromosomal **H. pylori** flaA gene by the cat-inactivated cloned gene copy via homologous recombination resulting in allelic exchange. Phenotypic characterization of the mutants demonstrated the absence of flagella under the electron microscope and the loss of bacterial motility. Immunoblots of cell lysates of the **H. pylori** mutants with an antiserum raised against the C-terminal portion of recombinant **H. pylori** major flagellin (FlaA) confirmed the absence of the 54 kDa FlaA protein. This efficient transposon shuttle mutagenesis procedure for **H. pylori** based on natural competence opens up new possibilities for the genetic assessment of putative **H. pylori** virulence determinants.

L7 ANSWER 11 OF 12 MEDLINE
AN 93273693 MEDLINE
DN 93273693
TI Cloning and genetic characterization of the **Helicobacter pylori** and Helicobacter mustelae flaB flagellin genes and construction of **H. pylori** flaA- and flaB-negative mutants by electroporation-mediated allelic exchange.
AU Suerbaum S; Josenhans C; Labigne A
CS Unite des Enterobacteries, Institut Pasteur, INSERM U199, F-75724 Paris, France..
SO JOURNAL OF BACTERIOLOGY, (1993 Jun) 175 (11) 3278-88.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-L08907; GENBANK-L08908
EM 199309
AB **Helicobacter pylori** is one of the most common human pathogens. It causes chronic gastritis and is involved in the pathogenesis of gastroduodenal ulcer disease and possibly gastric carcinoma. Helicobacter mustelae is a bacterium closely related to **H. pylori** that causes gastritis and ulcer disease in ferrets and is therefore considered an important animal model of gastric Helicobacter infections. Motility, even in a viscous environment, is conferred to the bacteria by several sheathed flagella and is regarded as one of their principal virulence factors. The flagellar filament of **H. pylori** consists of two different flagellin species expressed in different amounts. The gene (flaA) encoding the major flagellin has recently been cloned and sequenced. Here we report the cloning and sequencing of two highly homologous new flagellin genes from **H. pylori** 85P and H. mustelae NCTC 12032. The nucleotide sequence of the **H. pylori** gene proved that it encoded the second flagellin molecule found in **H. pylori** flagellar filaments. The genes were named flaB. The H. mustelae and **H. pylori** flaB genes both coded for proteins with 514 amino acids and molecular masses of 54.0 and 53.9 kDa, respectively. The proteins shared 81.7% identical amino acids. The degree of conservation between **H. pylori** FlaB and the **H. pylori** FlaA major flagellin was much lower (58%). Both flaB genes were preceded by sigma 54-like promoter sequences. Mapping of the transcription start site for the **H. pylori** flaB gene by a primer extension experiment confirmed the functional activity of the sigma 54 promoter. To evaluate the importance of both genes for motility, flaA- and flaB-disrupted mutants of **H. pylori** N6 were constructed by electroporation-mediated allelic exchange and characterized by Western blot (immunoblot) analysis and motility testing. Both mutations selectively abolished the expression of the targeted gene without affecting the synthesis of the other flagellin molecule. Whereas flaA mutants were completely **nonmotile**, flaB mutants retained motili

L7 ANSWER 12 OF 12 MEDLINE
AN 92105335 MEDLINE
DN 92105335
TI Serodiagnosis of **Helicobacter pylori**: comparison of enzyme-linked immunosorbent assays.
AU Talley N J; Newell D G; Ormand J E; Carpenter H A; Wilson W R; Zinsmeister A R; Perez-Perez G I; Blaser M J
CS Gastroenterology Research Unit, Mayo Clinic, Rochester, Minnesota 55905..
SO JOURNAL OF CLINICAL MICROBIOLOGY, (1991 Aug) 29 (8) 1635-9. Journal code: HSH. ISSN: 0095-1137.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199204
AB Enzyme-linked immunosorbent assays (ELISAs) have been developed to diagnose **Helicobacter pylori** infection. However, the methods are not standardized. We therefore prospectively evaluated the sensitivities and specificities of ELISAs developed in the United States and the United Kingdom in a study population comprising 41 consecutive symptomatic outpatients and 35 volunteers. At endoscopy, multiple biopsies were obtained for histology and culture and stained sections were graded for chronic gastritis, active chronic gastritis, and density of **H. pylori**. Serum samples were analyzed for **H. pylori** by ELISA. The first set of assays for immunoglobulin G (IgG) and IgA used a pool of sonicated isolates of **H. pylori** from five patients in the United States (antigen A). The second set of assays, developed in the United Kingdom, used three different antigens: antigen 1, an acid-extractable surface antigen; antigen 2, an acid-extractable antigen from an **aflagellate** variant; and antigen 3, a urease-containing fraction. Cutoff scores for positive results were determined a priori on the basis of previous serological studies. There was close agreement between histology and culture. In the study population, 36% of the individuals were **H. pylori** positive. The diagnostic value of the different ELISAs were highly comparable, and the crude antigens performed as well as the more purified antigens. The antigen A IgG had a sensitivity and specificity of 96 and 94%, respectively; the values for antigen 1 were 93 and 96%, respectively. The antigen A IgA and antigen 3 assays were the least sensitive tests.(ABSTRACT TRUNCATED AT 250 WORDS)